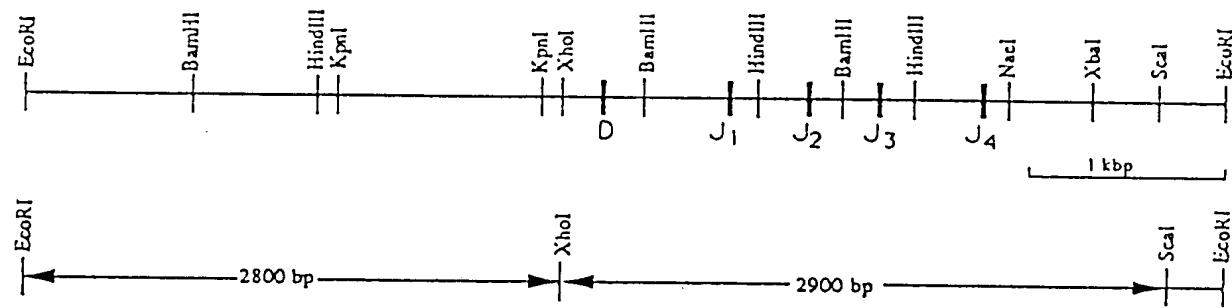


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Mouse Heavy Chain J Genes Inactivation Vector

(A) Targeted mouse heavy chain J genes



(B) Inactivation vector mDΔJ.Neo

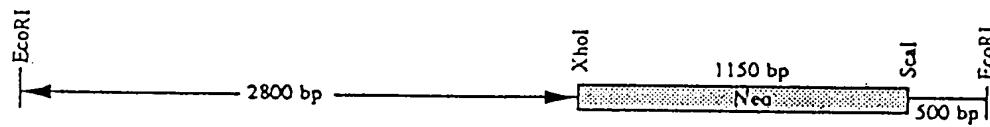
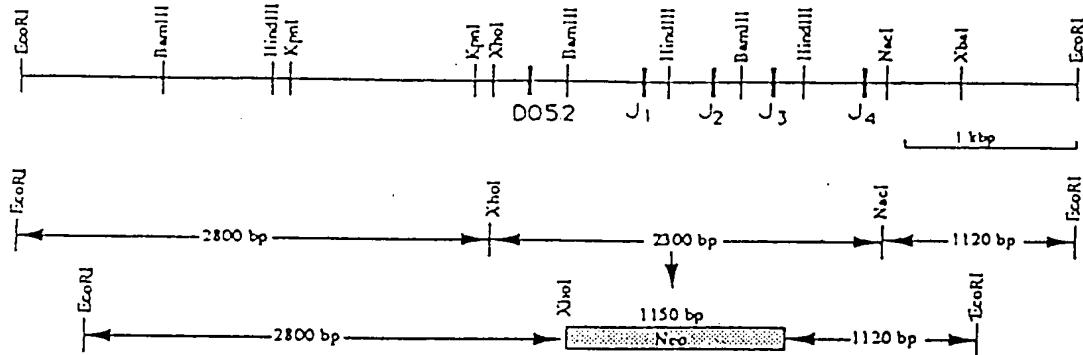


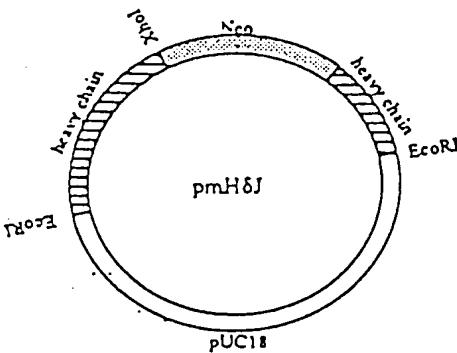
Figure 1

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(A) Targeted mouse heavy chain J genes

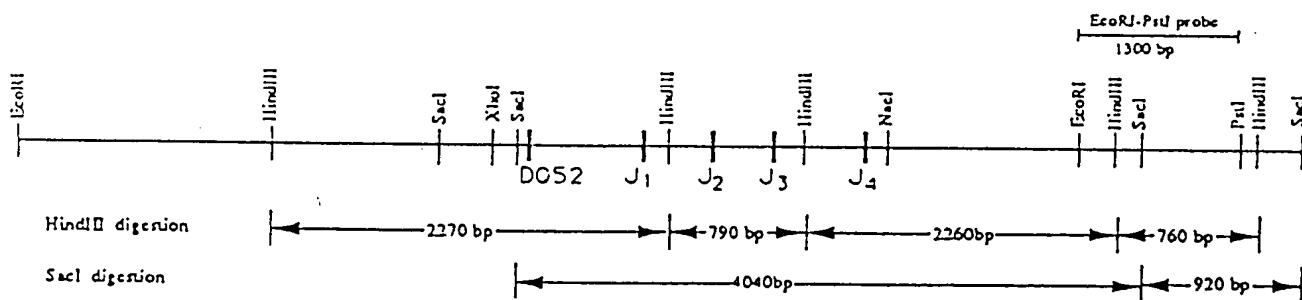


(B) Inactivation vector pmHδJ



(C) Southern analysis of pmHδJ-targeted ES colonies

Wild type ES cell genome



Targeted ES cell genome

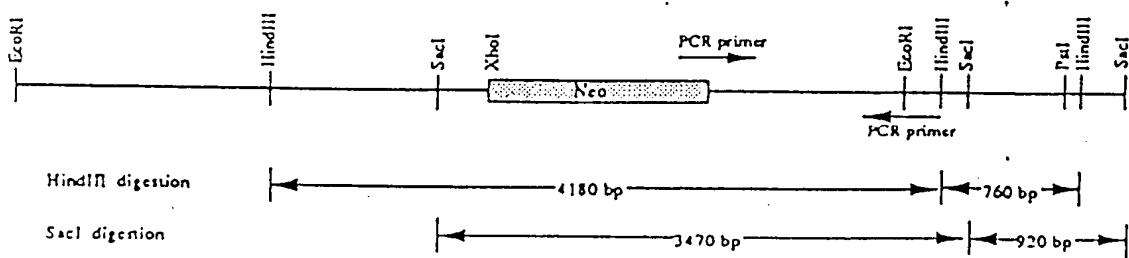
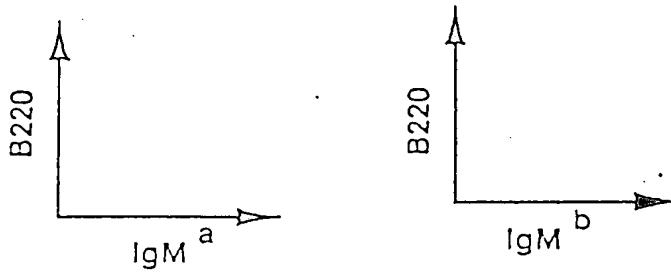


Figure 2

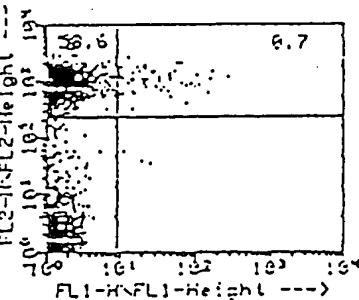
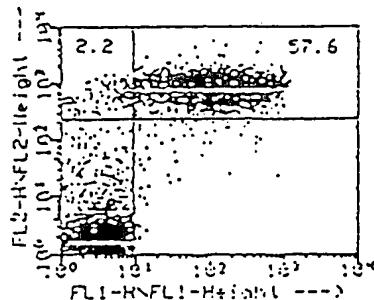
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J_H deletion blocks cell surface IgM expression



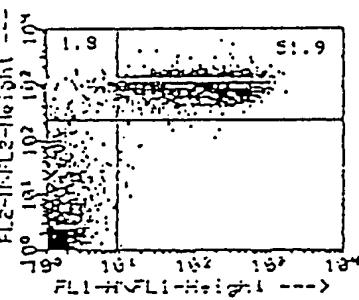
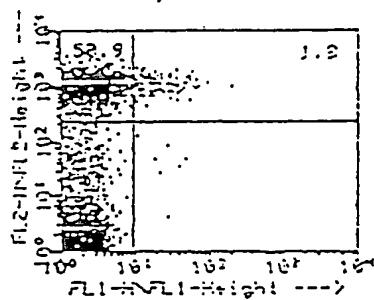
A.

a allotype



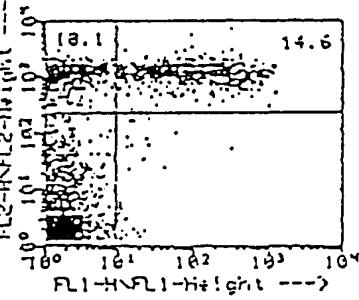
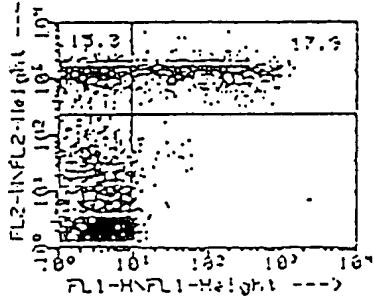
B.

b allotype



C.

a / b F1



D.

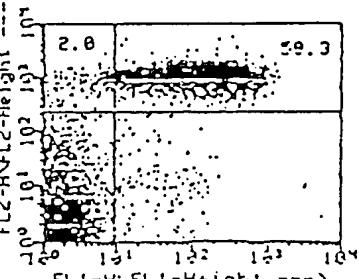
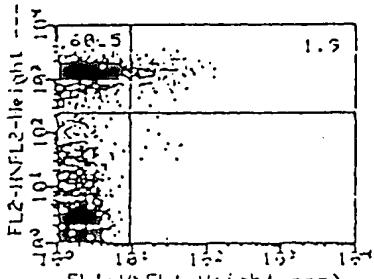
 $\Delta J_H / b$ F1

Figure 3

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Staining of peripheral blood lymphocytes with fluorescent anti-a allotype (A, D), anti-b allotype (B,E) or anti- β 220 (C,F). (A, B, C) JI-I-deletion homozygous mutant mouse 244-3-2/F2-7, (D) A allotype control mouse, (E) B allotype control (F) control mouse. The number in each panel indicates the percentage of cells stained with the specific antibody.

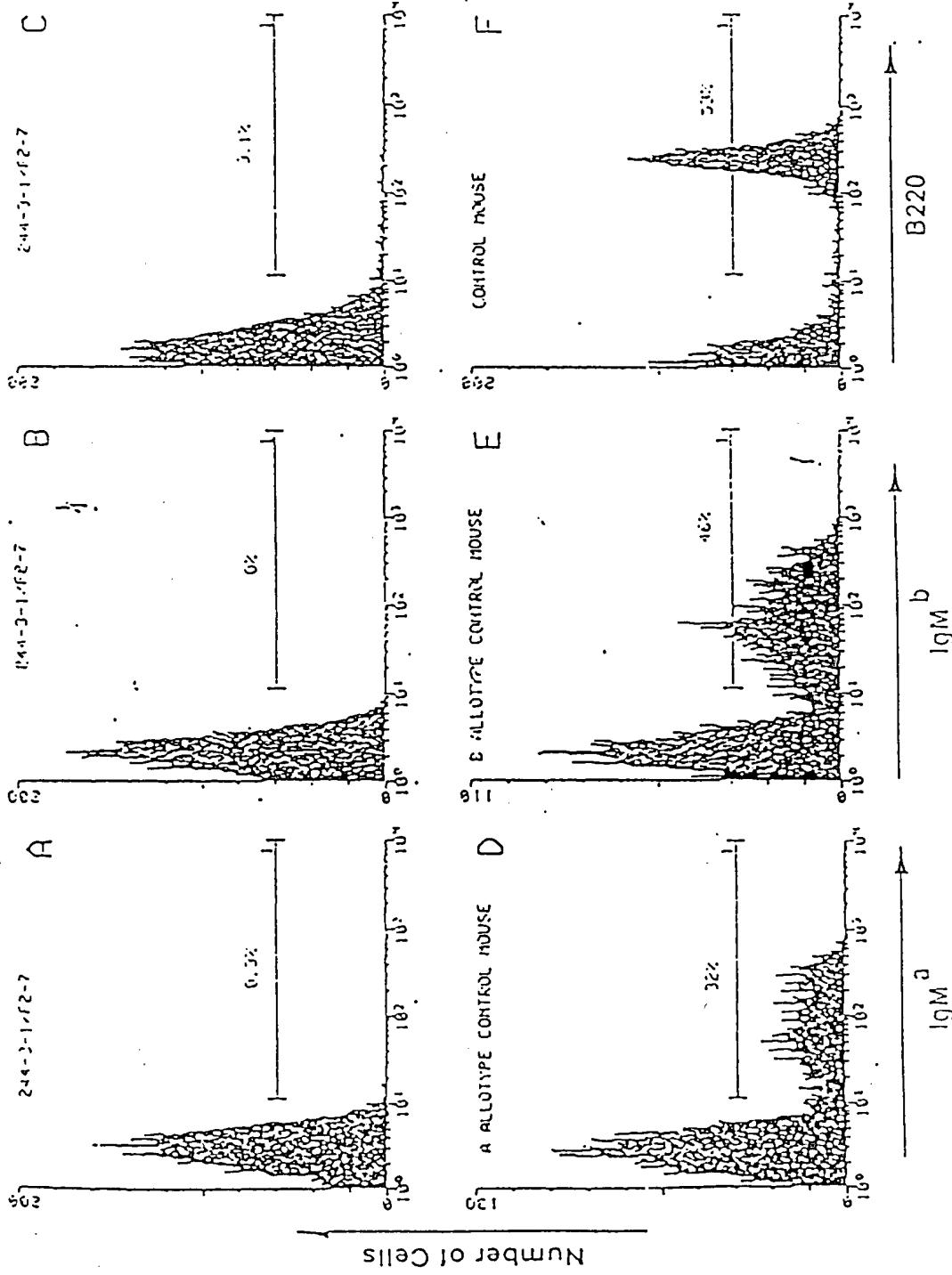


Figure 4

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INACTIVATION OF KAPPA CONSTANT REGION

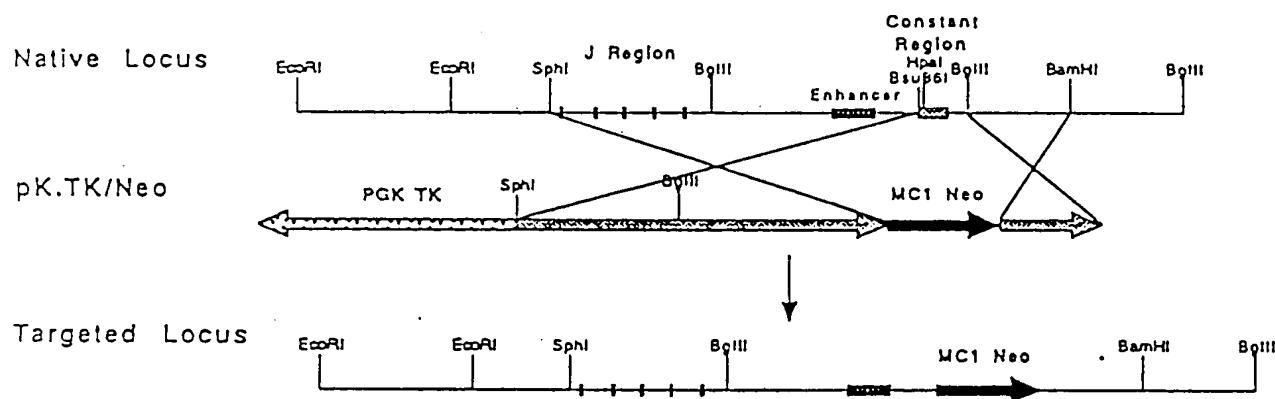


Figure 5

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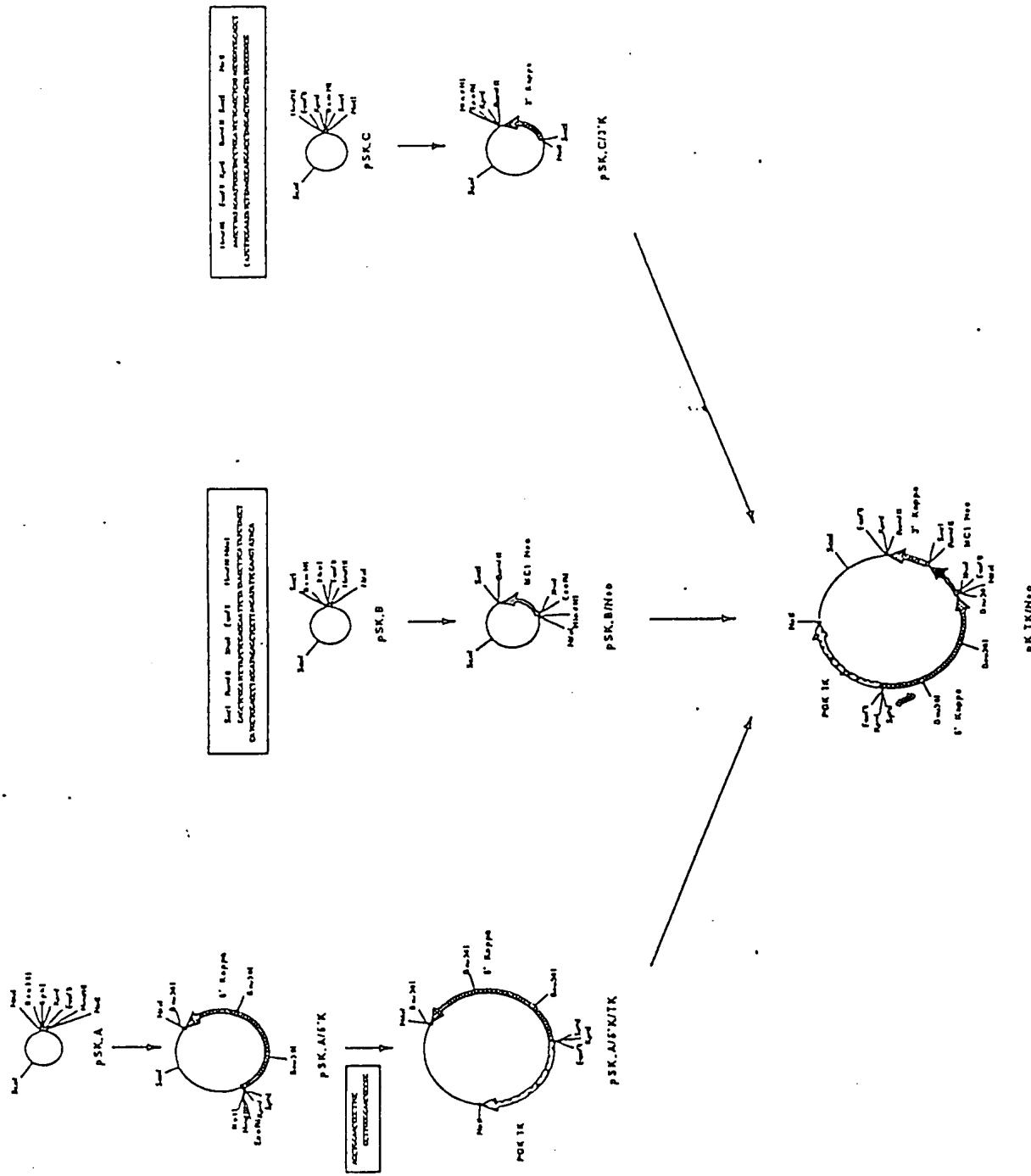
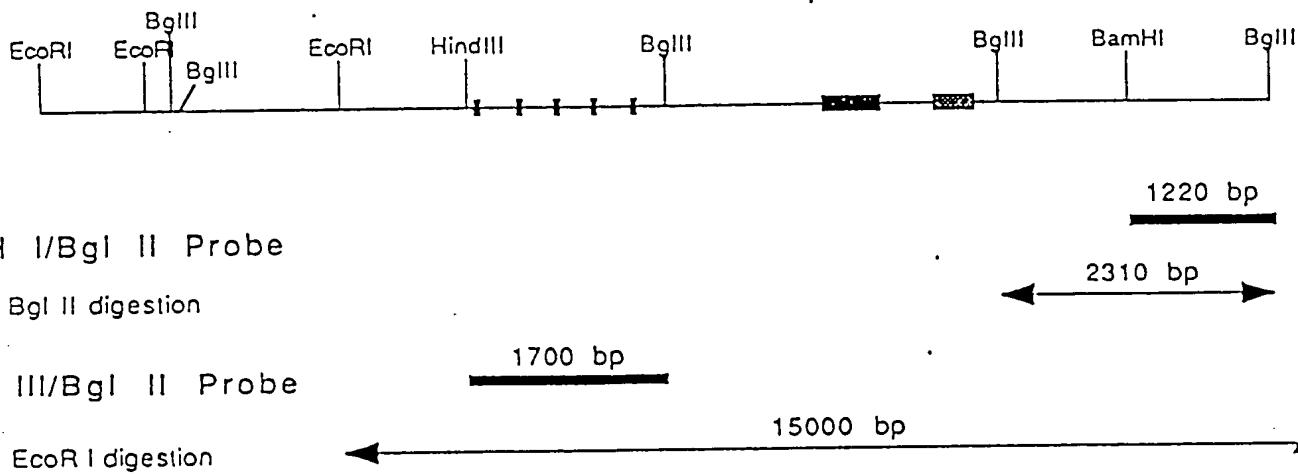


Figure 6

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SOUTHERN ANALYSIS OF LIGHT CHAIN C_K-TARGETED E14-1 CELLS

NATIVE ES CELL LOCUS



TARGETED ES CELL LOCUS

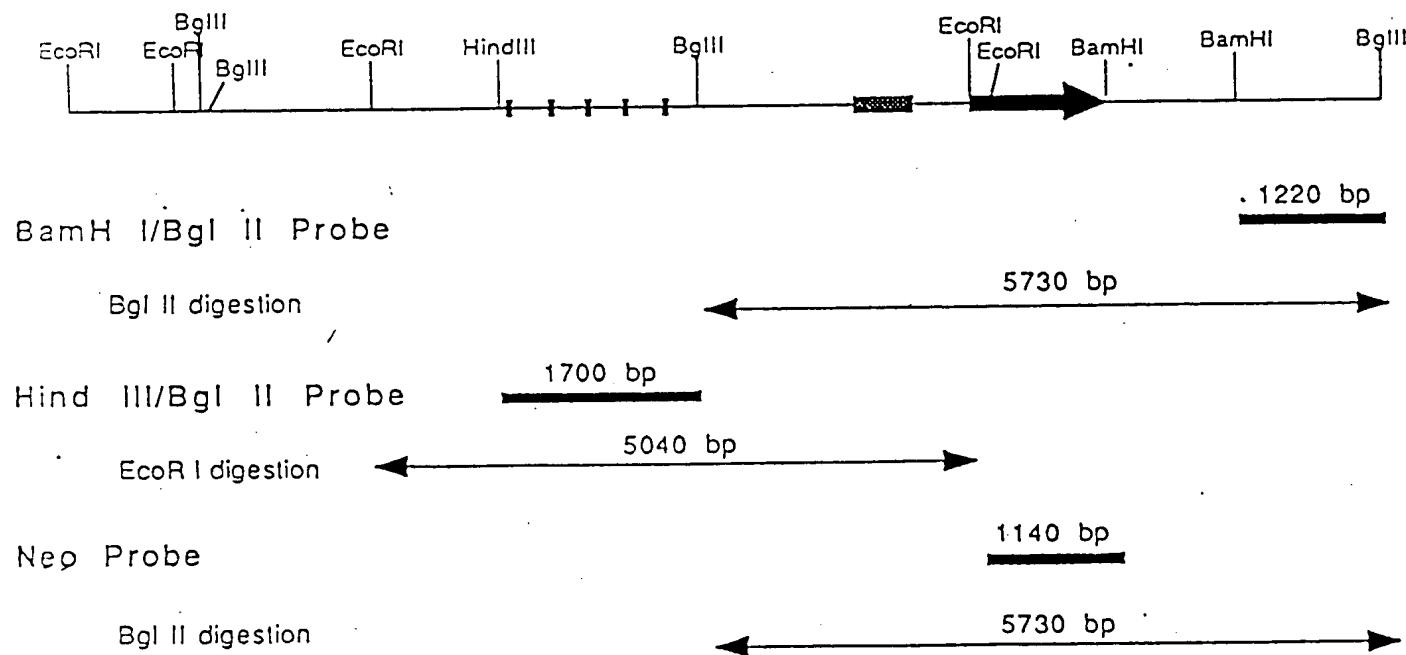
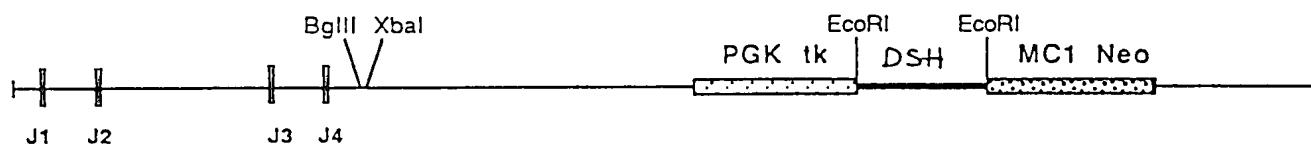


Figure 7

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KAPPA J/CONSTANT REGION INACTIVATION

J REGION KNOCKOUT VECTOR



TARGETING SCHEME

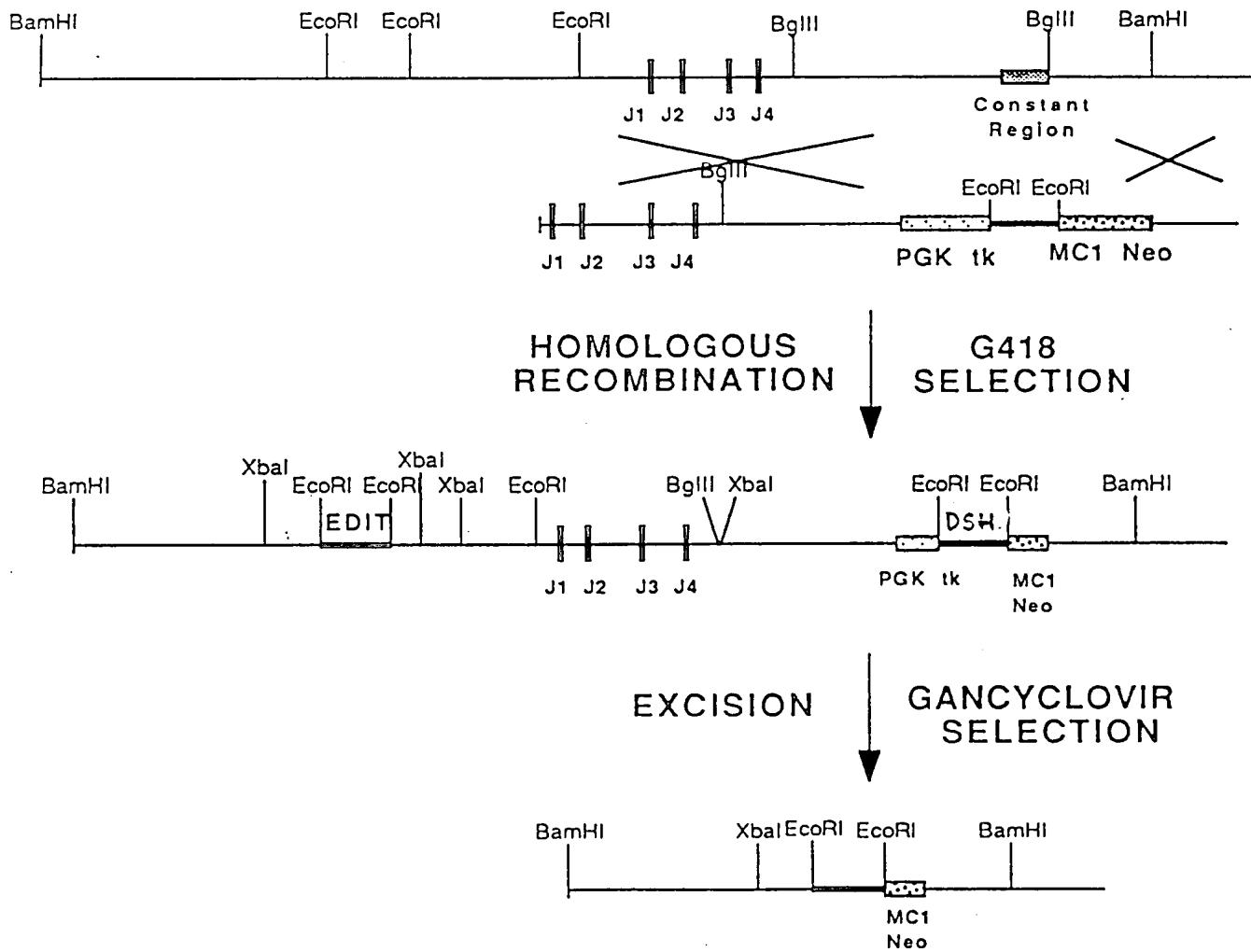


Figure 8

CONSTRUCTION OF KAPPA J/CONSTANT REGION DELETION VECTORS

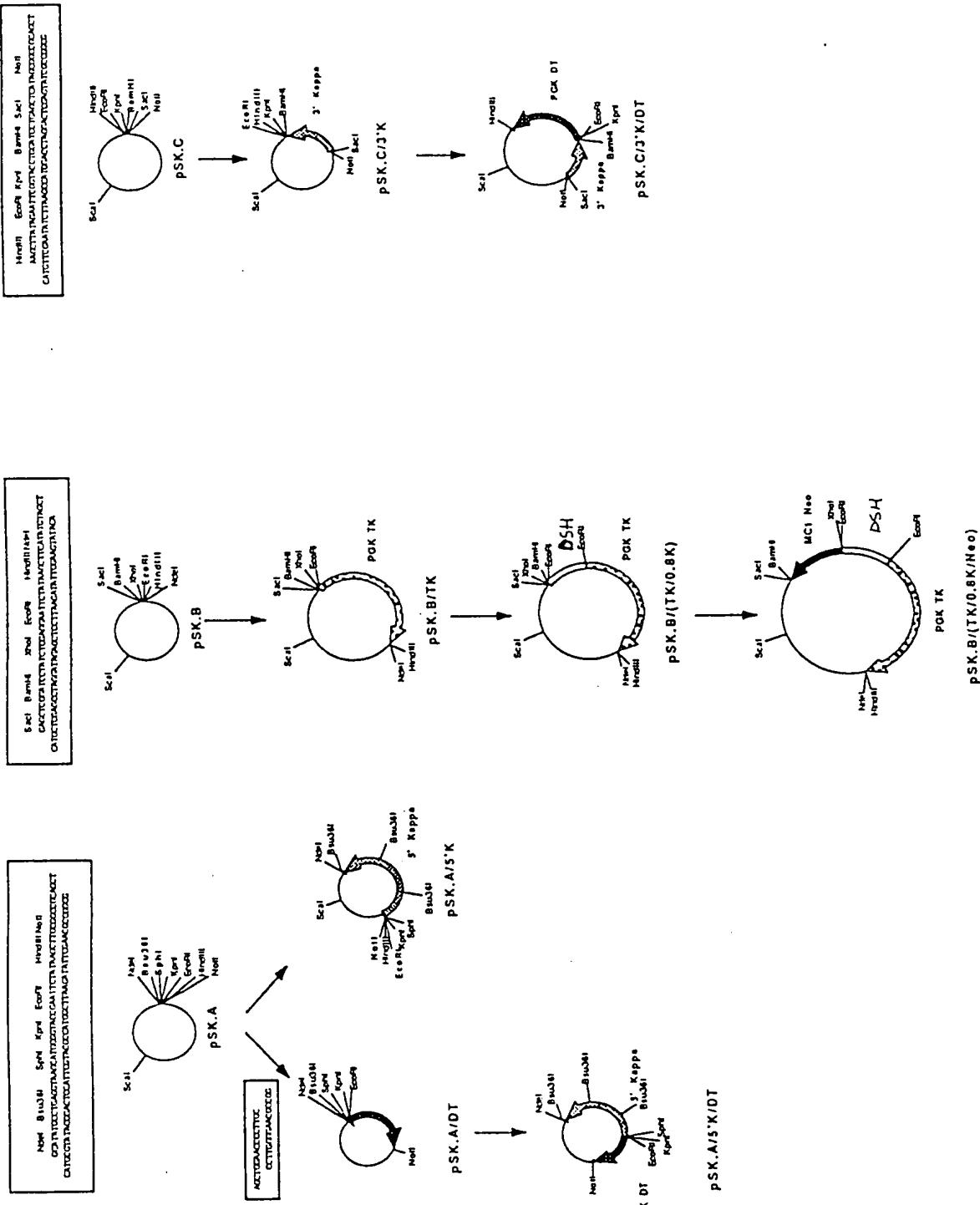


Figure 9

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KAPPA J/CONSTANT REGION DELETION VECTORS

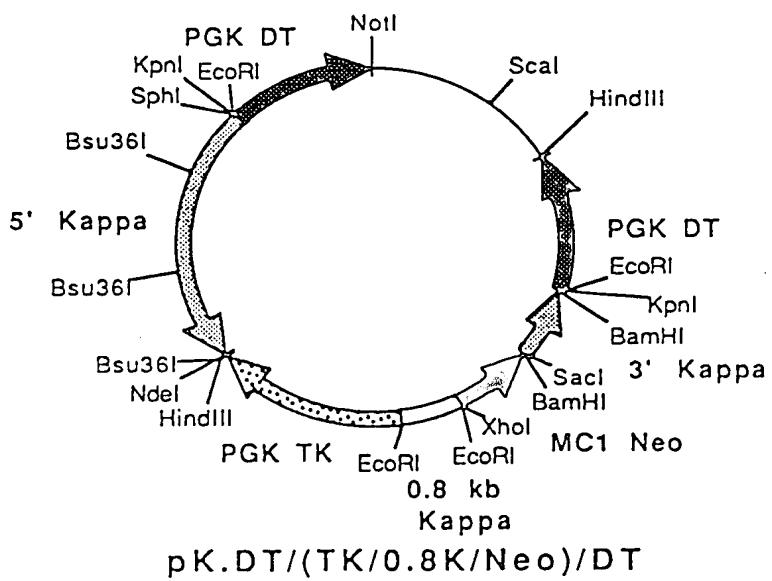
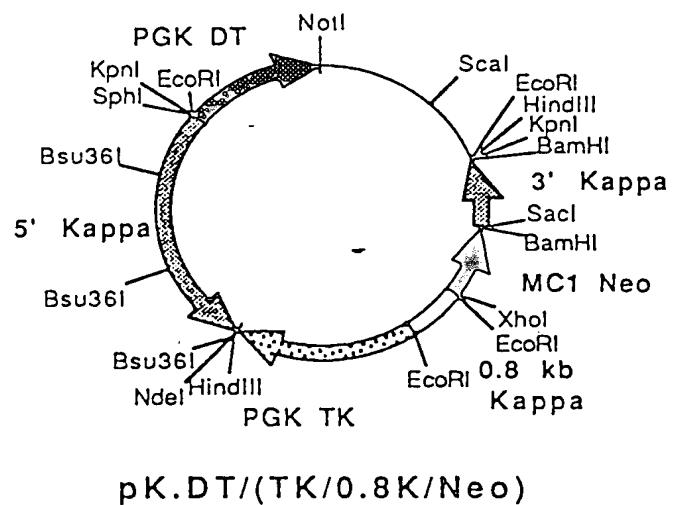
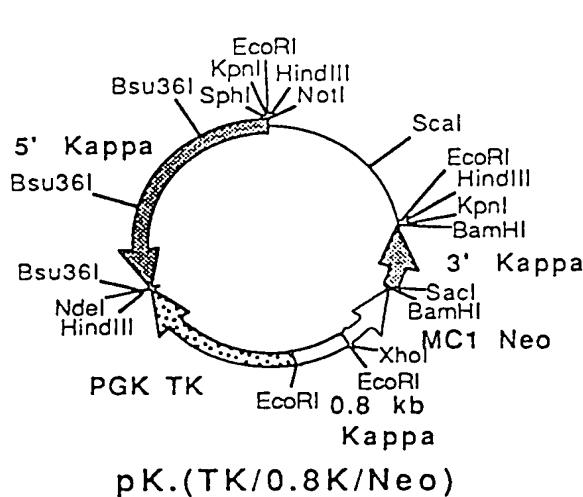
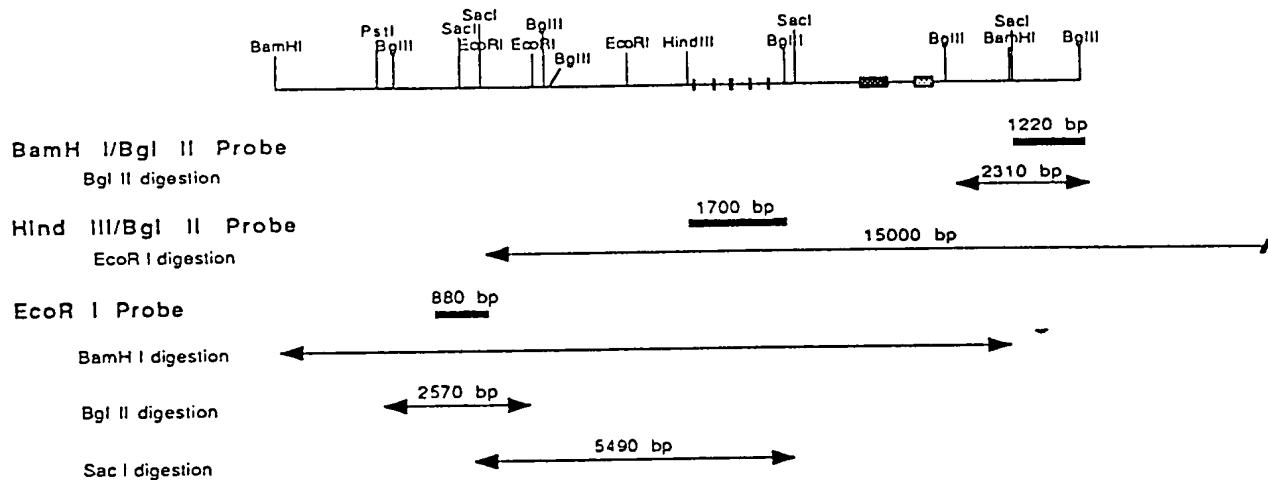


Figure 10

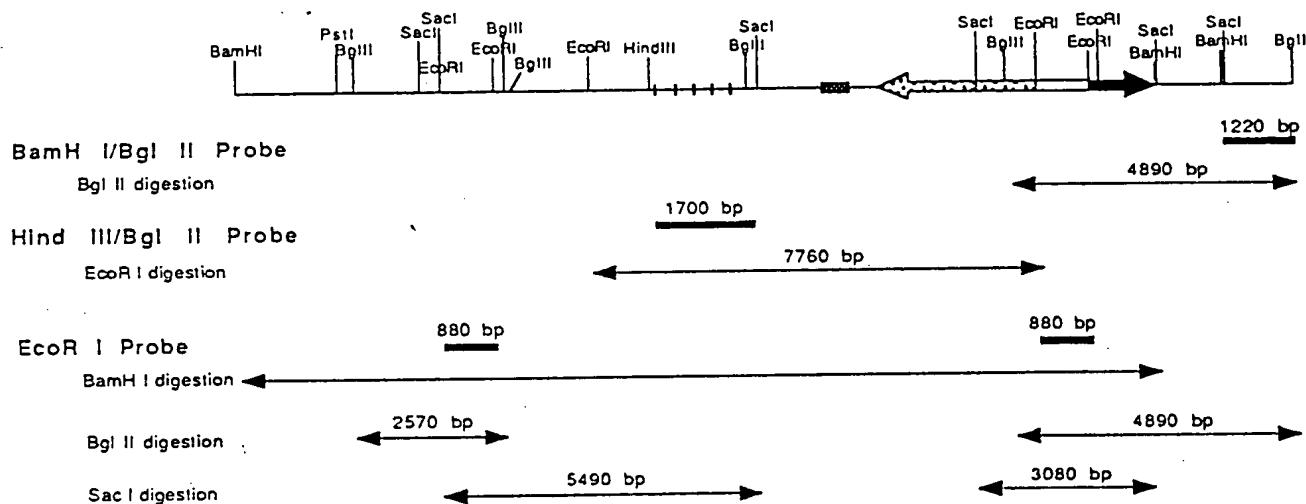
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SOUTHERN ANALYSIS OF LIGHT CHAIN $J\kappa/C\kappa$ -DELETED E14-1 CELLS

NATIVE ES CELL LOCUS



$C\kappa$ -TARGETED ES CELL LOCUS



$J\kappa C\kappa$ -DELETED ES CELL LOCUS

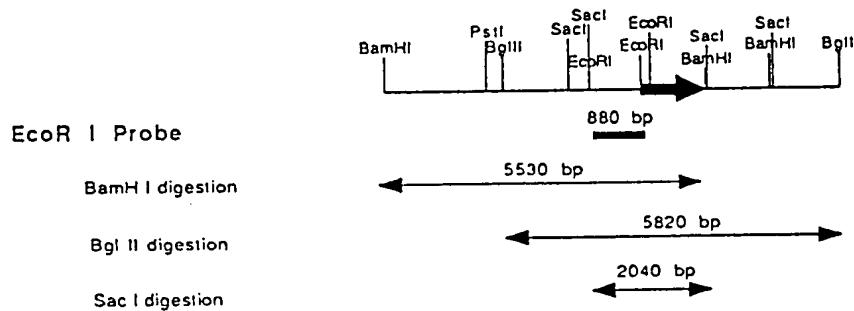


Figure 11

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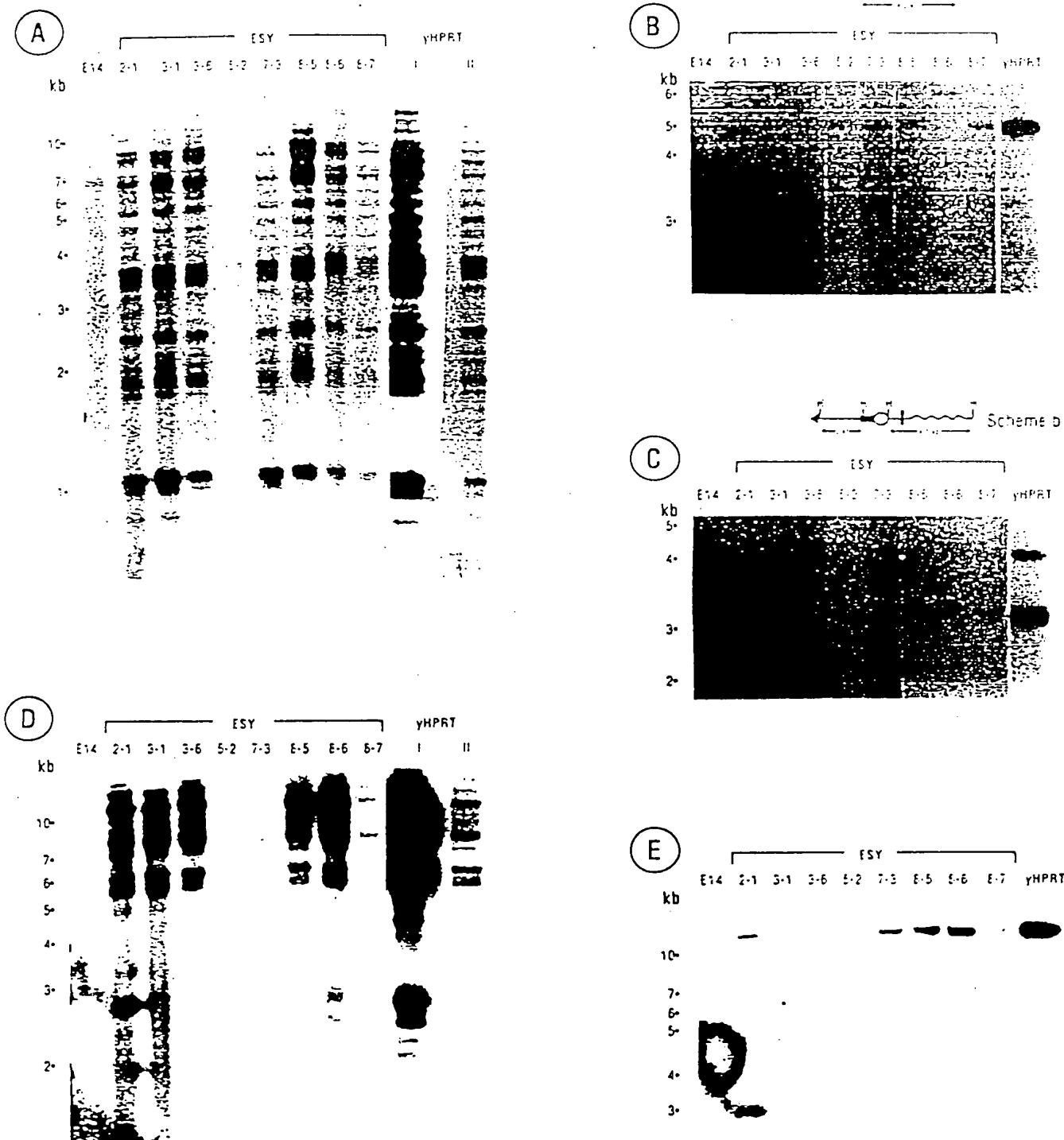


Figure 12

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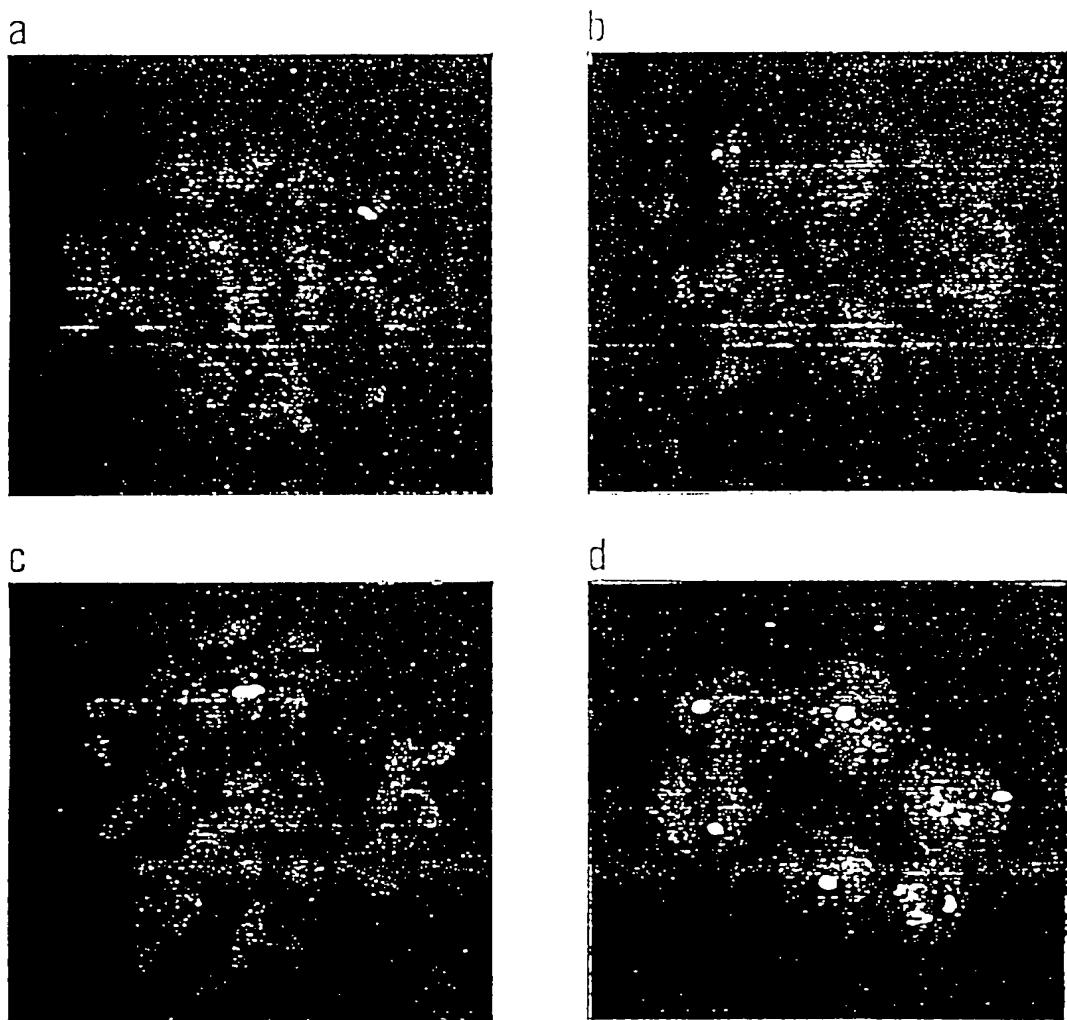


Figure 13

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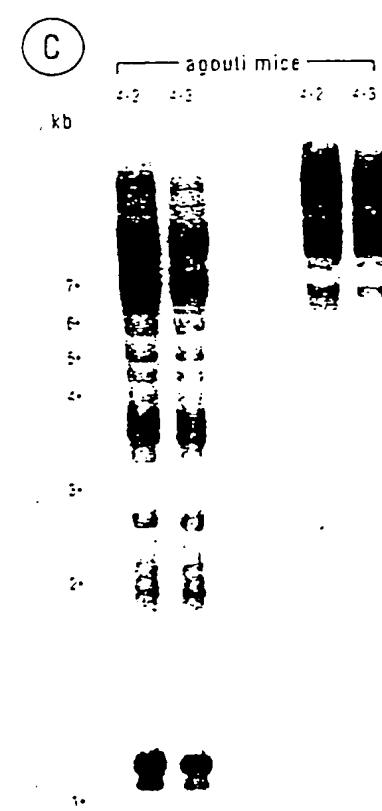
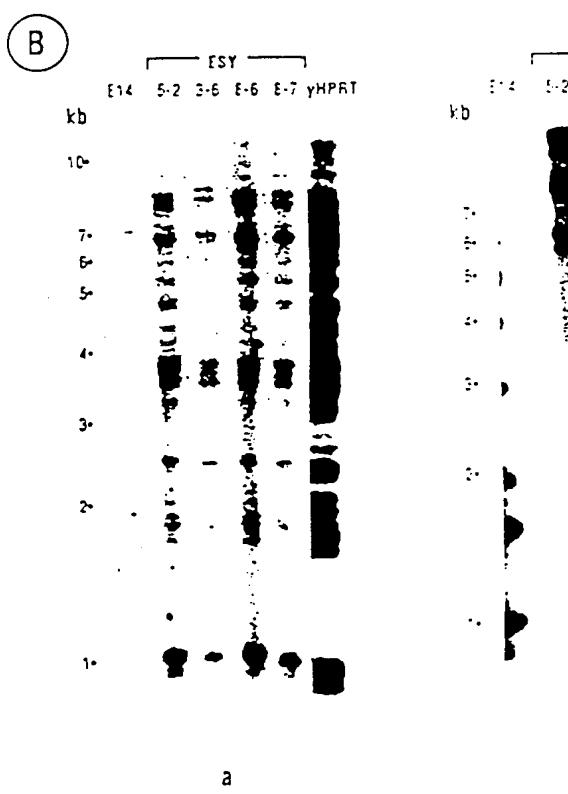
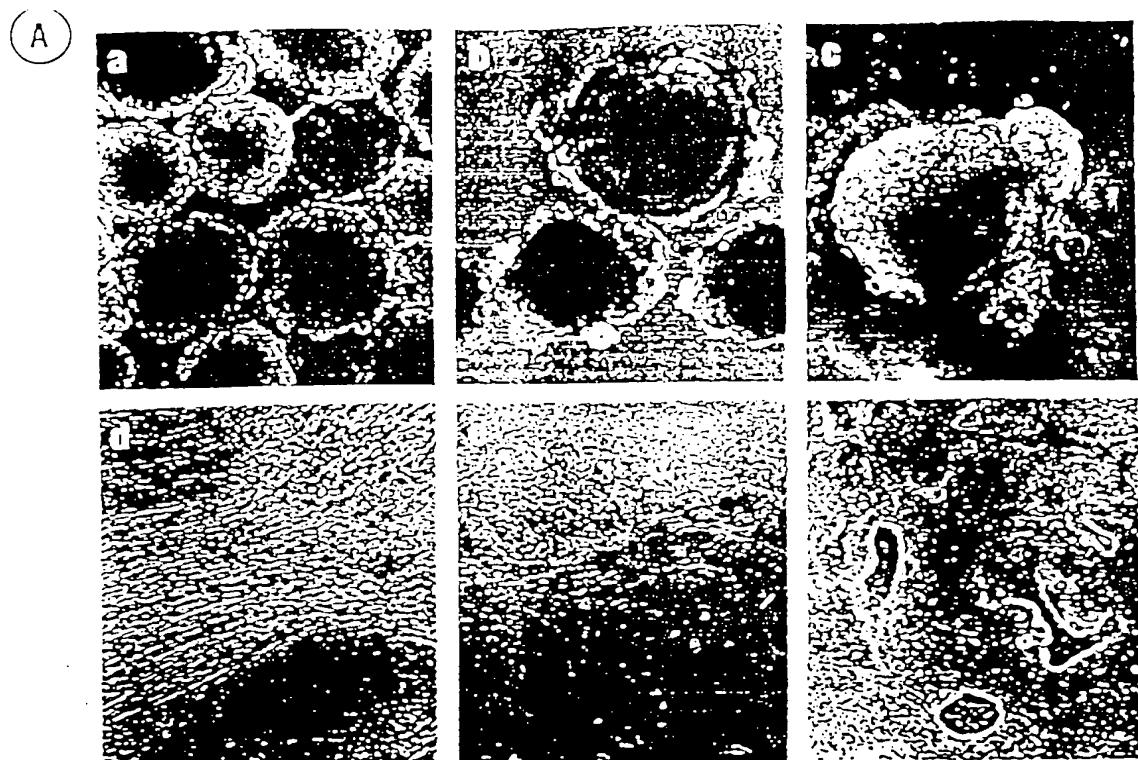


Figure 14

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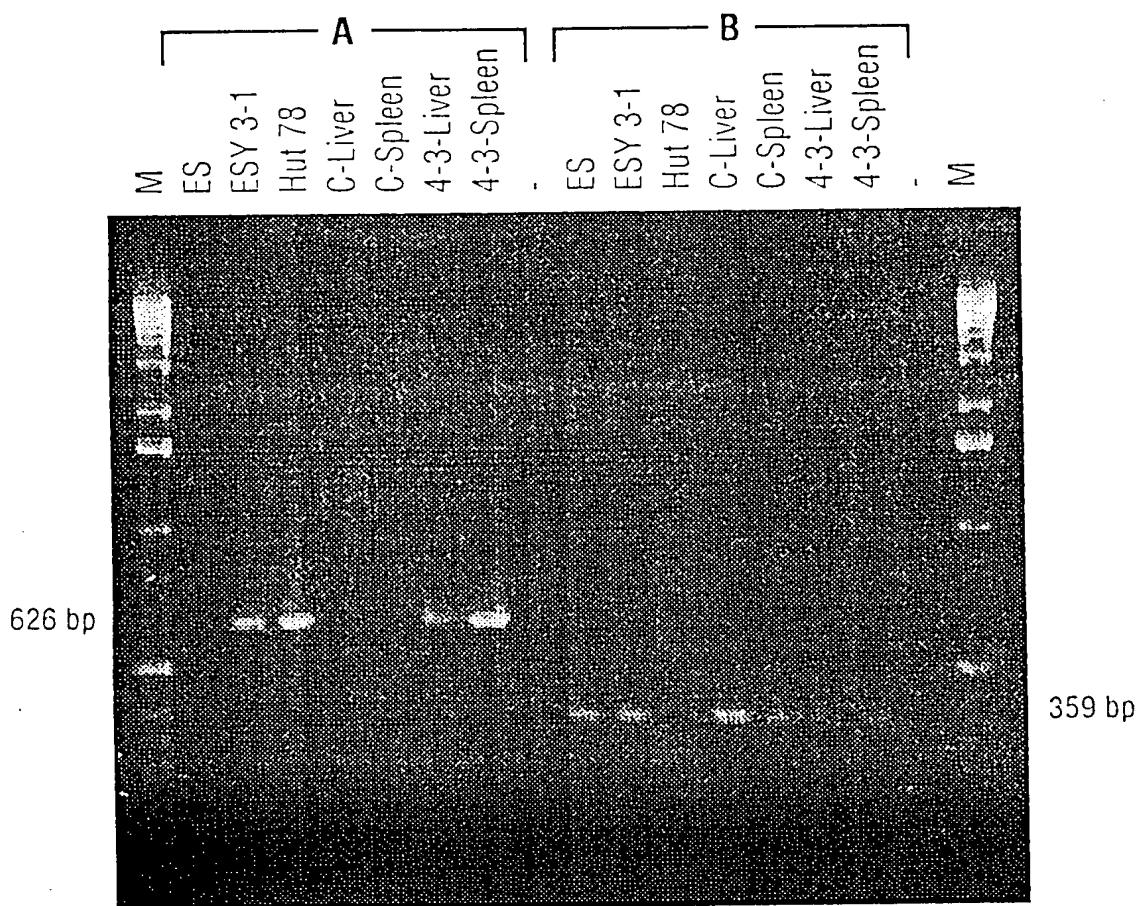
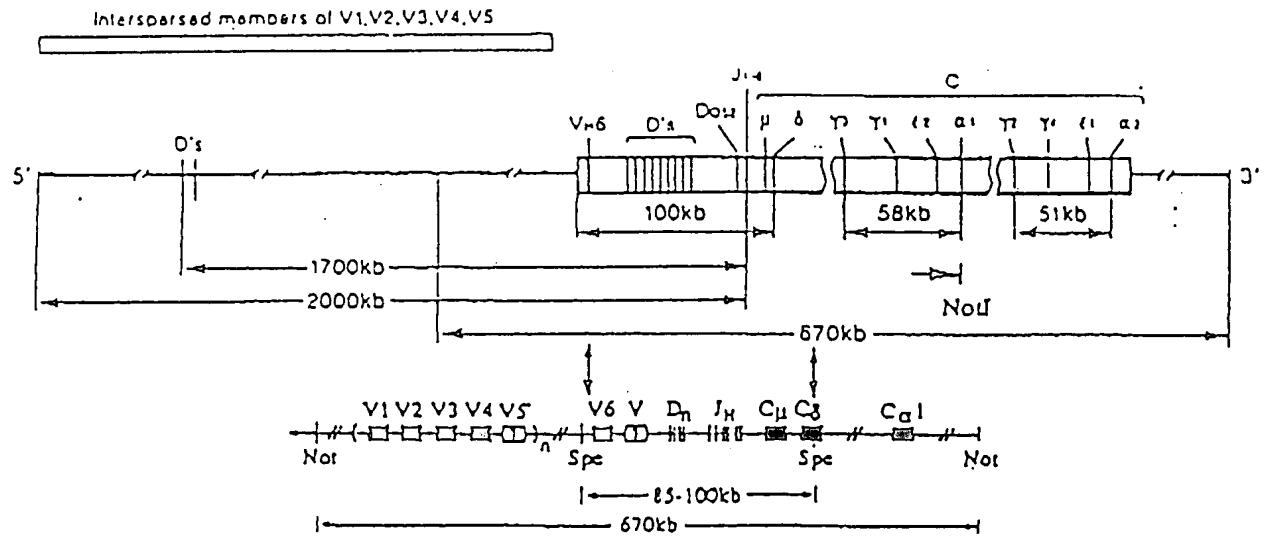
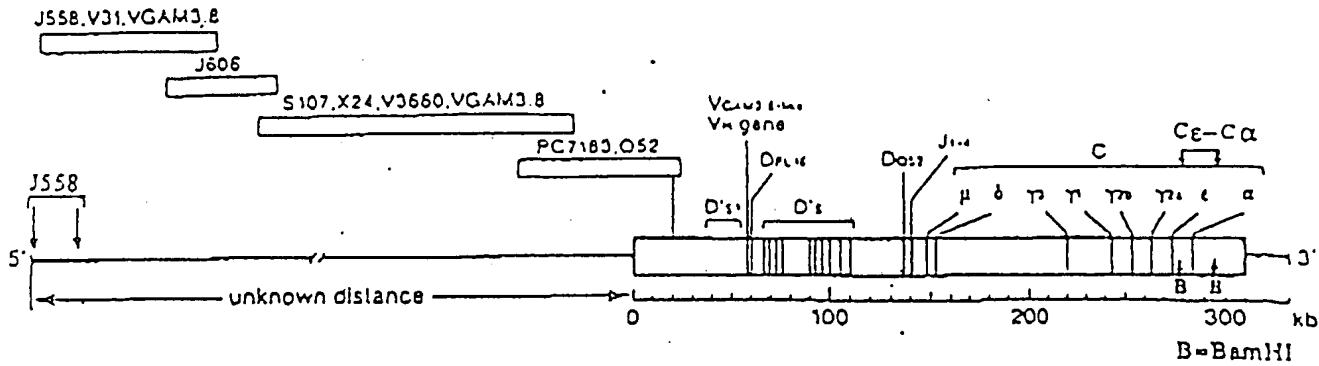


Figure 15

(A) Human heavy chain locus



(B) Mouse heavy chain locus



(C) Human heavy chain replacement YAC vector

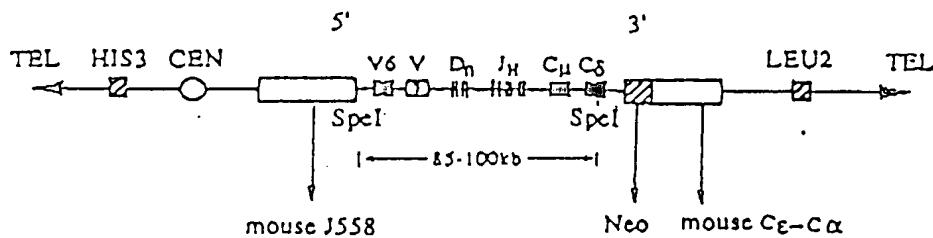


Figure 16

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Mouse Breeding Scheme

Cross IA.

heterozygous inactive Murine IgH
X

heterozygous inactive Murine IgK

MIgH (inactive) MIgK

MIgH MIgK

X

MIgH MIgK (inactive)
MIgH MIgK

▽

F1 (cross IA)

MIgH (inactive) MIgK (inactive)
MIgH MIgK

Cross I B.

heterozygous Human IgH
X

heterozygous Human IgK

MIgH MIgK HIgH

MIgH MIgK

X

MIgH MIgK HIgK
MIgH MIgK

▽

F1 (cross I B)

MIgH MIgK HIgH HIgK
MIgH MIgK

Cross II.

F1 (cross IA) x F1 (cross I B)

▽

F2 Quadruple Heterozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH MIgK

Cross III.

Intercross F2 mice

▽

F3 DOUBLE Homozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH (inactive) MIgK (inactive)

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MAMMALIAN HOST GENOTYPES

<u>Hetero- or Hemi-zygous Mice</u>	<u>Intercross Product Mice*</u>
I. <u>$\Delta mIgL$</u> <u>$mIgH$</u> <u>$mIgL$</u> <u>$mIgH$</u>	<u>$\Delta mIgL$</u> <u>$mIgH$</u> <u>$\Delta mIgL$</u> <u>$mIgH$</u>
II. <u>$mIgL$</u> <u>$\Delta mIgH$</u> <u>$mIgL$</u> <u>$mIgH$</u>	<u>$mIgL$</u> <u>$\Delta mIgH$</u> <u>$mIgL$</u> <u>$\Delta mIgH$</u>
III. <u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgH$</u> <u>$mIgL$</u> <u>$mIgH$</u>	<u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgH$</u> <u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgH$</u>
IV. <u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$mIgL$</u> <u>$mIgH$</u>	<u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u>
V. Animal I X Animal II	
<u>$\Delta mIgL$</u> <u>$mIgH$</u> <u>$mIgL$</u> <u>$\Delta mIgH$</u>	<u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u>
VI. Animal III X Animal V	
<u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgH$</u> <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u>	<u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgH$</u> and <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgH$</u> <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgH$</u> and <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgH$</u>
VII. Animal IV X Animal V	
<u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u>	<u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> and <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> and <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgH$</u>
VIII. Animal VI X Animal VII	
<u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u>	<u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u>
<u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u>	<u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> and <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> and <u>$\Delta mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgH$</u>
IX. Animal III X Animal IV	
<u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> <u>$mIgL$</u> <u>$mIgH$</u>	<u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> <u>$mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u>
X. Animal II X Animal IX	
<u>$mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> <u>$mIgL$</u> <u>$mIgH$</u>	<u>$mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> and <u>$mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> <u>$mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> and <u>$mIgL$</u> <u>$\Delta mIgH$</u> <u>$hIgH$</u>
XI. Animal I X Animal IX	
<u>$\Delta mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> <u>$mIgL$</u> <u>$mIgH$</u>	<u>$\Delta mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> and <u>$\Delta mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> <u>$\Delta mIgL$</u> <u>$mIgH$</u> <u>$hIgL$</u> <u>$hIgH$</u> and <u>$\Delta mIgL$</u> <u>$mIgH$</u> <u>$hIgH$</u>

*Not all possible genotypes from intercrosses are shown.

A = functionally inactive locus
m = mouse endogenous gene
h = human transgene
IgH = immunoglobulin heavy chain
IgL = immunoglobulin light chain